Human food flavor additives as bird repellents: I. Conjugated aromatic compounds

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Abstract: Avian repellents derived from natural products and human food flavorants may be less expensive to register under United States environmental pesticide registration requirements. However, one difficulty faced by workers attempting to target repellents for development is the need to screen large numbers of compounds for activity, as well as consideration of formulation and environmental constraints. In this study, we compare the bird repellent activity of aldehyde-based human food additives and compare the levels of activity with our previously elucidated model for structure–activity relationships (SAR) for bird repellents. We find that a previously elucidated SAR model for identifying acetophenone and anthranilate bird repellents is applicable to predicting the activity of aromatic aldehyde flavorants as well. In particular, of the nine flavorants tested, four, benzaldehyde, cinnamaldehyde, *o*-tolualdehyde, and *o*-anisaldehyde, warrant further consideration as bird repellents.

Keywords: bird; repellents; aldehydes; structure–activity; wildlife; management

1 INTRODUCTION

American and European societies are placing increasing importance on repellents rather than on population reduction for crop protection from birds.[1] Thus, discovery and development of effective repellents that render crops unpalatable is of general interest. Regrettably, most synthetic materials identified to date that deter birds have unacceptable toxic and environmental properties.[2–4] Frequently such compounds were designed as insecticides or fungicides, with their utility as bird repellents having been discovered only subsequently.[5–8]

Drawing upon a long history of exploiting the evolved chemical defenses of plants as sources of insect repellents,[9–12] recent studies on bird repellents have focused on identifying and quantifying the active ingredients derived from natural products.[13–15] The theory behind these efforts is that the evolved mechanisms of plant defenses are less likely to be acutely lethal or to have long-term negative environmental effects. Rather, plant defenses are thought to be more likely to modify behavior because they are sensory irritants or because they mediate avoidance learning.[16] This perception is reflected in the United States Environmental Protection Agency's move to exempt repellents derived from plant extracts that do not pose unreasonable adverse effects on the environment (section 25(b)(2) of the Federal Insecticide, Fungicide, and Rodenticide Act, FIFRA).[17] Many of these natural products or their synthetic analogues, besides having bird-repellent properties, are also used by humans as fragrances and flavors.[18,19] The presumption for exploring the repellent properties of these compounds is that they may achieve efficacy at low risk to non-target species and the environment.

Work in our laboratory has focused on elucidating the chemical properties of aromatic compounds, eg acetophenones, anthranilates, and benzoates, and their structural relationship to bird repellency.[20] The present study investigates the bird-repellent potential of several conjugated aromatic compounds commonly used as food flavors by humans[18] and relates the repellency of these compounds to previously elucidated qualitative structure–activity relationships. In particular, we previously showed the following features to be important for good primary bird repellents: (1) the molecule possesses benzene ring; (2) the molecule tends to be planar; (3) the molecule has a high degree of resonance, and (4) the molecule has a high degree of basicity. Thus, steric effects, acidic substituents, and extreme delocalization of lone pairs of pi-electrons (as occurs with *meta* isomers and aromatics multiply substituted with electron-donating groups) all tend to detract from repellency.[20–24]

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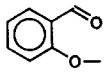
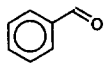
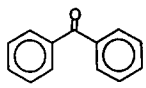
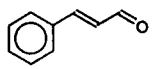
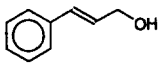
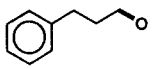
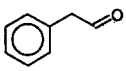
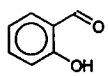
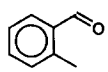
Table 1. Summary of the concentrations used and human perceptual characteristics of potential bird repellents

	[CAS] Compound	FDA[a]	FEMA[b]	Odor[c]	Irritation[c] Taste[c]	Use[d]	ppm[e]
	135-02-4 *o*-anisaldehyde	121.1164		Floral	Sweet, bitter		
	100-52-7 benzaldehyde	GRAS	2127	Almond	Bitter	1–7	36–840
	119-61-9 benzophenone	121.1164	2134	Rose		1, 3–5	0.5–2
	14371-10-9 cinnamaldehyde	GRAS	2286	Spicy	Pungent, burn	1, 3–9	8-4, 900
	104-54-1 cinnamic alcohol	121.1164	2294	Floral	Bitter	1–7	5–720
	104-53-0 dihydro-cinnamaldehyde	121.1164	2887	Floral/hyacinth	Balsamic, burn	1, 3–6	1–6
	122-78-1 phenylacetaldehyde	121.1164	2874	Fruit/green	Pungent, bitter	1, 3–5, 7	0.7–87
	90-02-8 salicylaldehyde	121.1164	3004	Nut	Pungent	1–5, 7–8	0.6–18
	529-20-4 *o*-tolualdehyde	121.1164	3068	Almond	Bitter	1, 3–7	8–430

[a] FDA: Code of Federal Regulations.
[b] FEMA: Industry food and flavor codex.
[c] Odor, Irritation, and Taste are qualities perceived by humans.
[d] Examples of food products containing the compounds: 1 = non-alcoholic beverages, 2 = alcoholic beverages, 3 = ice cream, 4 = candy, 5 = baked goods, 6 = gelatins/puddings, 7 = chewing gum, 8 = condiments, 9 = meats.
[e] ppm is the reported range of concentrations used for various food products.

2 EXPERIMENTAL METHODS

2.1 Study subjects

European starlings (*Sturnus vulgaris* L) were chosen as test animals because previous experiments have shown them to be good models of avian sensitivity. Starlings were captured at Sandusky, Ohio and transported to the Monell Center. Starlings were individually caged ($61 \times 36 \times 41$ cm) under a 12:12 h light:dark cycle for at least a two-week adaptation period and given free access to Purina Flight Bird Conditioner (Purina Mills, St. Louis, MO), water and oyster shell grit (United Volunteer Aviaries, Nashville, TN). Capture, maintenance, and experimental protocols were carried out in compliance with guidelines set forth by the Institutional Animal Care Committee.

2.2 Stimuli

We selected a series of conjugated aromatic compounds commonly used as human food flavor additives (Table 1). Based on our qualitative model, electron donation (pi orbital) from substituents to the benzene ring is considered to be an important feature contributing towards bird repellency.[8] Hence, the potency of aromatic repellents containing simple substituent fragments is predicted to be ordered as $NH_2 > OCH_3 > CH_3 > OH$. In this study we predicted potency of the following compounds to be ranked as: benzaldehyde > *o*-anisaldehyde > salicylaldehyde > *o*-tolualdehyde. Because resonance is also a contributory factor in the potency of a repellent,[21] disrupting the resonance pattern by placing a saturated bond structure between the benzene ring and the electron-withdrawing fragment should diminish the potency of a repellent (ie phenylacetylaldehyde < benzaldehyde and phenylacetylaldehyde < cinnamaldehyde). Moreover, we predicted that moving the electron-withdrawing group (EWG) further from the benzene ring while maintaining resonance should result in diminished potency of the repellent. Thus, cinnamaldehyde should be a less effective repellent than benzaldehyde. Alcohol fragments placed within the EWG tend to diminish the repellent potency of compounds.[20,24] Thus, we predicted that dihydro-cinnamaldehyde would be less potent than cinnamaldehyde. Eliminating the EWG, hence resonance, and adding an acidic functionality would presumably result in a compound that is not repellent, eg cinnamic acid. Finally, increasing the size of the substituent along a freely rotating axis should cause steric hindrance, and this

should eliminate repellent activity of a compound, cf benzaldehyde and benzophenone. Prior to testing, we determined the water solubilities of the compounds using standard spectrophotometric techniques.[9–24] This information proved useful for making concentration-corrected, cross-chemical comparisons.

2.3 Effects of maximum water-soluble concentrations of compounds on fluid consumption

Besides the intrinsic activity of a molecule, avoidance responding can vary as a function of the concentration of the compound tested. Therefore, the maximum water-soluble concentration of compounds was tested as an overall index of repellency potential, ie maximum potency. The experimental design utilized a standard one-bottle drinking assay[21] and consisted of an adaptation/assignment phase and a test phase. During the adaptation/assignment, birds were presented with tap water in calibrated drinking tubes on each of five days. Water intake was recorded every two hours for six hours, and then the graduated tubes were replaced with standard water bottles. Overnight, birds were permitted *ad libitum* access to food and water. Starlings were randomly assigned to one of 10 groups ($n = 6$ per group), and equality among groups for total water intake was verified by one-way analysis of variance (ANOVA). For this test, baseline water consumption during the 6-h monitoring period across all groups was 17.6 ml (± 0.3 SEM).

Groups were randomly assigned to one of 10 treatments (Table 1) and fluid intake was monitored for every two hours for a total of six hours. The maximum capacity for repellency was evaluated by comparing the avoidance response of a specific compound at its maximum water-solubility concentration (up to a maximum concentration of 5 mg ml^{-1}) relative to the avoidance response to an equimolar concentration of two well-described bird repellents, methyl anthranilate[22] and *o*-aminoacetophenone.[21] The maximum water-soluble concentrations presented to starlings reflect a saturated avoidance response, ie maximum potency. It is unlikely that all of the test compounds mirror the concentration–response curve of methyl anthranilate. We previously showed that the shape of the concentration–response curve varies as a function of molecular structure.[20] Nonetheless, maximum potency is a useful index for initial screening of repellents because compounds that do not match activity levels of previously determined repellent standards are unlikely to be considered active in more detailed or alternative assays.

2.4 Effects of equimolar concentration of compounds on food consumption

The intrinsic activity of a molecule can vary as a function of the number of molecules that access mediating sensory receptors. Thus, presenting birds with repellents in liquid form maximizes the likelihood that the repellent will gain access to these receptors. However, most applications apply repellents to the surface of foods. Because birds have low salivary flow, and because repellents may be bound to food substrates, the number of molecules accessing mediating sensory receptors will be lower in circumstances when the repellent is applied to food. Thus, it takes a higher concentration of compound to achieve a given level of repellency when the compound is applied to food relative to when it is dissolved in water. Nonetheless, because most repellents are designed to deter feeding it is worthwhile to consider efficacy of these compounds when they are applied to food.

Briefly, 60-mM solutions of compounds were prepared in ethyl alcohol. Solutions were then sprayed onto the surface of maintenance chow (50 ml solution:100 g chow), and the alcohol was allowed to evaporate under a hood overnight.[25] On a percentage by weight basis, the range of concentrations tested across compounds was 0.3–0.4% (wt/wt). This concentration range corresponds to the 50% reduction in feed consumption by starlings presented with food treated with methyl anthranilate.

The methods of adaptation and group assignment were identical to those outlined in the first experiment with the following exceptions. Ten groups of four birds per group were used in the test. After being food-deprived at 1700 h the evening prior to being tested, starlings were tested once for two hours for each of four mornings. During the test, a single food cup containing 25 g of food was presented to the birds. The difference in weight from the pre- and post-test period, adjusted for spillage and weight changes attributable to atmospheric humidity (as determined by monitoring pre- and post-test weight changes of three isolated food cups) was taken as an index of repellency. Food consumption by birds in each of the treated food groups was compared by contrast analysis to the food consumption by control birds, ie the profile for food consumption across days was compared to that of the isolated control. If no significant day effects were observed, the overall control v. treatment comparison of food intake collapsed over days was considered. A two-factor fixed effects ANOVA was used to test for hour (a repeated measure) and stimulus effects on fluid consumption. Post-hoc comparisons were made using Tukey's Honestly Significant Difference method ($P < 0.05$).

3 RESULTS

3.1 Repellency of flavorants in a drinking assay

More-soluble compounds were more repellent (Fig 1). With the exceptions of benzophenone, phenylacetylaldehyde and salicylaldehyde, the test compounds were as potent as the equimolar concentration of the well-described bird repellent, methyl anthranilate. However, when compared to the more potent bird repellent, *o*-aminoacetophenone, only benzaldehyde had the same equimolar potency. This assay does not reflect the shapes of the concentration–response curves of the test compounds, nor does it indicate

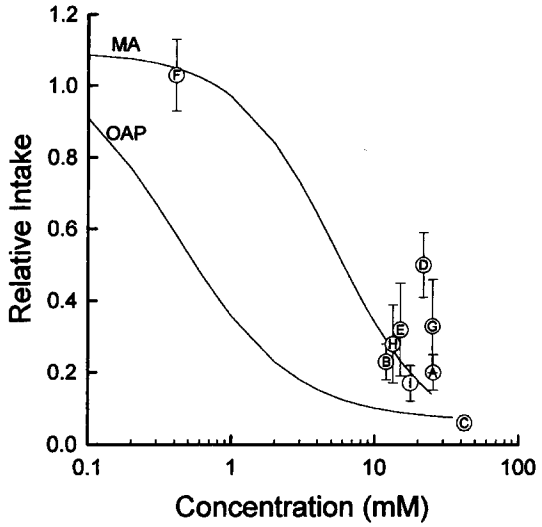


Figure 1. Relative intake of test solutions by starlings as a function of concentration. Each point depicts the maximum repellent effect (potency) of the test stimulus as a function of the compound's maximum water-solubility concentration. Codes are: [A] cinnamyl alcohol, [B] cinnamaldehyde, [C] benzaldehyde, [D] phenylacetylaldehyde, [E] hydro-cinnamaldehyde, [F] benzophenone, [G] salicylaldehyde, [H] *o*-tolualdehyde, [I] *o*-anisaldehyde. Capped vertical bars are ± SEM. For reference the concentration–response curves for two well-described bird repellents, methyl anthranilate (MA) and *o*-amino acetophenone (OAP) are included (solid lines).[21,22]

how structure of the compounds may influence the concentration-specific response. But the assay does serve to screen the compounds for their maximum repellent effect against known repellents of equal concentration. Thus, with the exception of benzophenone, phenylacetylaldehyde and salicylaldehyde, all other test compounds would be recommended for additional study as candidate repellents.

3.2 Repellency of flavorants in a feeding assay

Three of the flavorants were repellent to starlings in the context of one-cup feeding experiments (Fig 2). The pattern for consumption by starlings of food treated with benzaldehyde differed from that seen for untreated food ($F = 11.37$, $df = 1,27$, $P = 0.002$). Benzaldehyde suppressed food intake for the first day, but on subsequent presentations intake did not differ from control levels. Similarly cinnamaldehyde most strongly suppressed consumption on the first day of the test, and only weakly on subsequent days. Both are highly volatile compounds. Thus, there remains the possibility that on days 2 to 4 there was less of these substances remaining on the food relative to the first day of the test. Also, benzaldehyde is a notoriously unstable compound. Thus, the weaker effect of this

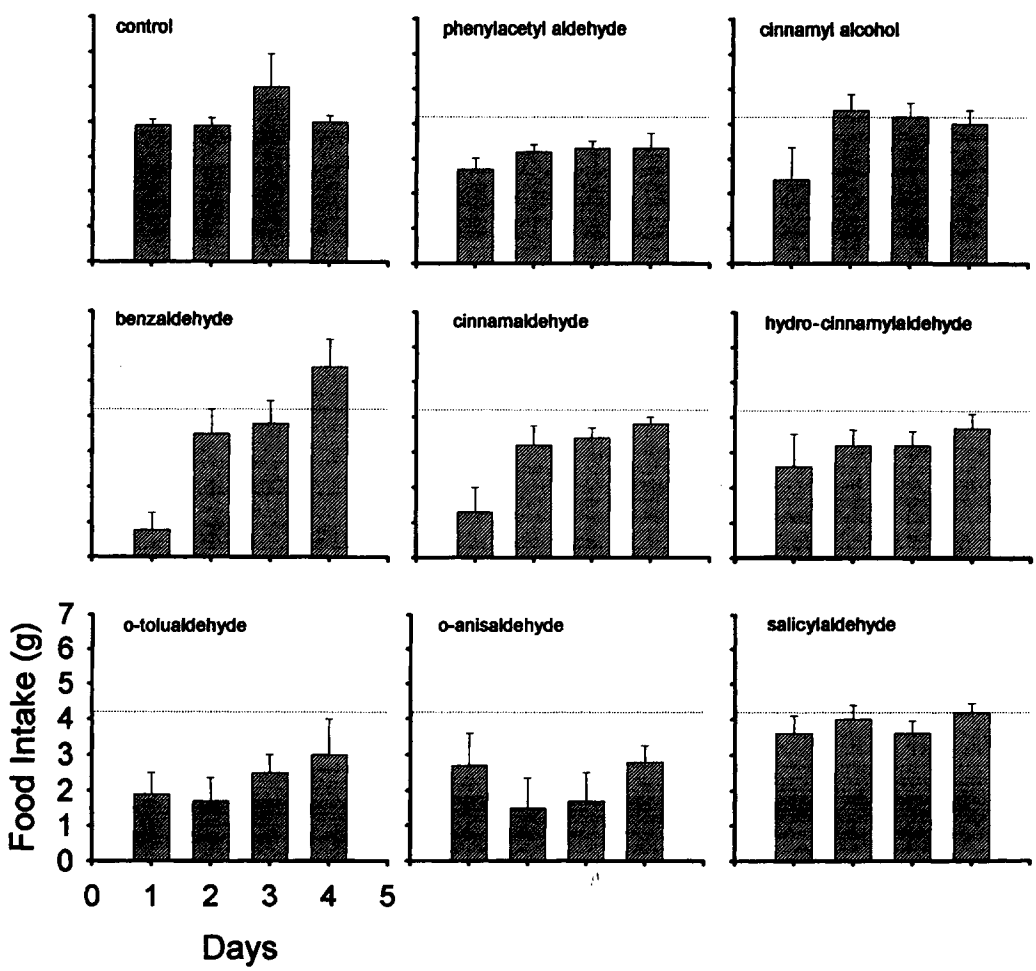


Figure 2. Consumption of food treated with test stimuli by starlings as a function of time. The horizontal dotted line depicts the average food intake of the control group. The capped vertical bars depict ± SEM.

substance could simply reflect degradation over the course of the study. Relative to controls, there were no differences in the pattern of food consumption across days for either *o*-tolualdehyde or *o*-anisaldehyde ($P=0.915$, 0.217, respectively). Thus, these compounds uniformly suppressed consumption relative to the intake observed for controls ($F=6.63$, $P=0.016$ and $F=7.37$, $P=0.011$, $df=1,27$, respectively). None of the other compounds reduced consumption of treated feed relative to untreated feed intake ($P>0.225$).

4 DISCUSSION

The maximum suppressive effect of the flavorants dissolved in water matched the level of avoidance observed for equimolar concentrations of the well-known bird repellent, methyl anthranilate. However, that does not mean that the diversity of structures of aldehydes were all equal in their repellent effect. In all likelihood the maximum water-soluble concentrations presented to starlings reflect a saturated avoidance response, ie maximum potency. However, it is unlikely that all of the test compounds mirror the concentration–response curve of methyl anthranilate. Clark and Shah[20] showed that the shape of the concentration–response curve varies as a function of molecular structure. Nonetheless, maximum potency is a useful index for initial screening of repellents because compounds that do not match activity levels of previously determined repellent standards are unlikely to be considered active in more detailed or alternative assays.

The structure–activity relationships of the compounds are better elucidated in the feeding assays. We previously showed that the *ortho* position was important to the activity of substituted benzene compounds, eg acetophenones and anthranilates.[21] In the same study, we also showed that the electron-donating potential of the *ortho* substituent enhanced repellency, ie amino > methoxy > hydroxy substitution. We observed the same pattern for the aldehydes tested in this study. Methyl (eg *o*-tolualdehyde), methoxy (eg *o*-anisaldehyde), and no substitution at the ortho position (eg benzaldehyde) all caused food avoidance. Hydroxy substitution (eg salicylaldehyde) produced no repellent effect. Moving the ketone group away from the benzene ring also decreased the repellent effect of the molecule (of benzaldehyde v phenylacetylaldehyde or cinnamaldehyde). This decrease may be owing to resonance (ie electronic effects) on the benzaldehyde substructure itself,[21] or to steric effects owing to the longer alkene chain.[22] Adding an alcohol to the alkene chain reduces activity as well (eg cinnamyl alcohol, cinnamic acid) perhaps by reducing the electronegativity of the attached carbon.[15,20,22]

5 CONCLUSIONS

As a group, the flavorings tested hold some potential for further study as bird repellents, though they may not be as potent as acetophenones or anthranilates.[21–23] The advantage of further considering these or similarly structured conjugated aromatic compounds is their frequent use as human food additives. Such compounds may have larger sets of pre-existing data on toxicological effects and thereby reduce the initial cost outlays in any potential registration effort. Furthermore, identification of individual compounds with activity could lead investigators to consider the natural source of the agents. Consideration of natural extracts as repellents under FIFRA section 3 registration may prove less expensive to a prospective registrant because of relaxed data requirements of the US EPA for natural-product-based repellents. However, even this approach will still be evaluated on a case-by-case basis.

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